PA NT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 June 2001 (06.06.01)	
International application No. PCT/GB00/03837	**Applicant's or agent's file reference** P7082WO CTH
International filing date (day/month/year) 05 October 2000 (05.10.00)	**Priority date** (day/month/year) 05 October 1999 (05.10.99)
Applicant SLINGSBY, Jason et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 March 2001 (20.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Zakaria EL KHODARY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HARDING, Charles, Thomas
D Young & Co.
21 New Fetter Lane
London EC4A 1DA
ROYAUME-UNI

Date of mailing (day/month/year)
01 December 2000 (01.12.00)

Applicant's or agent's file reference
P7082WO CTH

IMPORTANT NOTIFICATION

International application No.
PCT/GB00/03837

International filing date (day/month/year)
05 October 2000 (05.10.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
ROHL, Jonathan
Oxford BioMedica (UK) Limited
Medawar Centre
Robert Robinson Avenue
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Oxford OX4 4GA
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
ROHLL, Jonathan
Oxford BioMedica (UK) Limited
Medawar Centre
Robert Robinson Avenue
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Oxford OX4 4GA
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
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Facsimile No.: (41-22) 740.14.35

Authorized officer
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PATENT COOPERATION TR Y

11

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P7082WO CTH		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/03837	05/10/2000	05/10/1999
International Patent Classification (IPC) or national classification and IPC C12N15/867		
Applicant OXFORD BIOMEDICA (UK) LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/03/2001	16.01.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Brenz Verca, S Telephone No. +49 89 2399 7702

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03837

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-67 as originally filed

Claims, No.:

1-50 as received on 19/12/2001 with letter of 18/12/2001

Drawings, sheets:

1/18-18/18 as originally filed

Sequence listing part of the description, pages:

1-146, filed with the letter of 30.11.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03837

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 45-49.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 45-49.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03837

1. Statement

Novelty (N)	Yes:	Claims	1-34, 40-44
	No:	Claims	35-39, 50
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-44, 50
Industrial applicability (IA)	Yes:	Claims	1-39, 42-44, 50
	No:	Claims	

2. Citations and explanations
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB00/03837

Section III (Non-establishment of opinion)

Current claims 45-47 were not present in the application as initially filed, consequently no international search report in their respect has been established (ISR Box I.2) and accordingly they are not subject of an international preliminary examination (Rule 66.1(e) PCT).
Since current claims 48 and 49 are dependent of the unsearched claims 45-47, no opinion can be established for their subject matter.

Section V (Reasoned statement)

Reference is made to the following documents mentioned in the International Search Report:

D1: VANIN E. F. ET AL.: 'Development of high-titer retroviral producer cell lines by using Cre-mediated recombination.' JOURNAL OF VIROLOGY, vol. 71, no. 10, 1997, pages 7820-7826, ISSN: 0022-538X cited in the application

D2: KARREMAN S. ET AL.: 'ON THE USE OF DOUBLE FLP RECOGNITION TARGETS (FRTS) IN THE LTR OF RETROVIRUSES FOR THE CONSTRUCTION OF HIGH PRODUCER CELL LINES' NUCLEIC ACIDS RESEARCH, vol. 24, no. 9, 1 May 1996 (1996-05-01), pages 1616-1624, ISSN: 0305-1048 cited in the application

D3: IWAKUMA T. ET AL.: 'SELF-INACTIVATING LENTIVIRAL VECTORS WITH U3 AND U5 MODIFICATIONS' VIROLOGY, vol. 261, no. 1, 15 August 1999 (1999-08-15), pages 120-132, ISSN: 0042-6822

D4: BOAST K. ET AL.: 'CHARACTERIZATION OF PHYSIOLOGICALLY REGULATED VECTORS OF THE TREATMENT OF ISCHEMIC DISEASE' HUMAN GENE THERAPY, vol. 10, no. 13, 1 September 1999 (1999-09-01), pages 2197-2208, ISSN: 1043-0342

1. Novelty (Article 33(2) PCT)

1.1 Claims 35-39 are not novel for the reasons explained under point 4.3.

1.2 Claim 50 is not novel because it refers to any not further specified "method" "substantially described" in the description, including those described elsewhere and incorporated by reference (description p. 67, lines 14-15), thus including methods

well known in the art.

2. Inventive step (Article 33(3) PCT)

2.1 With regard to inventive step, **D1** is considered to be the closest prior art. It discloses a method to generate high titre retroviral producer cell lines by using Cre/loxP-mediated insertional recombination into a favourable preexisting site in the genome of the parental packaging cell line. Said favourable preexisting site is an integrated provirus carrying as a transgene an expression cassette for a selection marker flanked by two loxP sites located between the two identical viral LTRs. After that such a parental (or master) cell line has been selected for high level LTR-driven expression of the marker gene, the selection marker cassette is excised via transient expression of Cre recombinase, thus leaving back a single loxP site flanked by the viral LTRs. Any expression cassette for a gene of interest can be inserted by cre-mediated recombination into this site, thus the favourable high level expression selected in the master producer cell line can be obtained for any derived producer cell line. The efficiency of the process of recombinational insertion is sufficient to allow the generation of multiple new producer lines without the addition of antibiotic resistance genes (D1: abstract and 1st paragraph of discussion section on p. 7824). D1 thus already provides as technical effects the advantages (ii) and (iii) alleged by the applicant on page 9 of the description.

2.2 The difference from D1 is that the applicant generates by the stable cell line producer technology either a) high titre retroviral vectors with regulatable expression, b) self-inactivating (SIN) vectors or c) high titre conventional retroviruses with a replaced 3'-LTR. SIN vectors could be produced in prior art only by using transfection-based transient expression systems. Retroviral vectors with regulatable expression were difficult to obtain at high titre from stable producer cell lines, because the proviral RNA is expressed from the 5'-LTR which upon integration is identical to the original 3'-LTR containing the regulatable promoter elements.

2.3 The objective problems to be solved were thus the following: to provide a method to produce by the stable cell line producer technology the following types of vectors:

1) high titre regulatable retroviral vectors (REG)

2) SIN retroviral vectors (SIN)
3) Conventional retroviral vectors (CON)

2.4 The applicant solves the problem by introducing via Cre/lox-mediated recombination into a favourable preexisting site (see 2.1) of a master producer cell line (in the following: MPC) a new 3'-LTR (mentioned as "construct LTR" in the claims) different from the preexisting provirus LTR(s). In the so derived producer cell line (in the following: DPC), this construct LTR will assume the function of a 3'-LTR in the regulated or SIN retroviral genomes transcribed from the provirus generated by this recombinational insertion, but also conventional retroviruses with a replaced 3'-LTR can be produced.

2.5 Making abstraction from the clarity problems in the present set of claims, in the light of the description, inventive step is not acknowledged for the following reason:

In addition to the disclosure mentioned under 2.1, **D1** mentions that the approach to retroviral producer cell line production described therein "provides a means to generate high-titre SIN vectors" (D1: last paragraph of the discussion section). This sentence is a clear suggestion of a concrete application of the producer cell technology described in D1 and is thus a sufficient incentive for the skilled person to immediately put some effort in designing the features needed for this purpose. Although the Applicant alleges that this disclosure is not enabling because no worked example is presented in D1 on this subject, said modifications are within the skills of a person working in the field of retroviral vectors and knowing well their biology: Indeed, at the date of filing, it is reasonable to expect that the average skilled person working in the field of retroviruses had the basic knowledge that i) the U3 and U5 region are duplicated during reverse transcription of the retroviral RNA, creating identical LTRs having the structure U3-R-U5 at both ends of the proviral DNA, ii) the transcription of further retroviral RNAs from the integrated proviral DNA is directed from the U3 region in the 5'-LTR, iii) the principle of a classic SIN vector is that, a modified, transcriptionally inactive U3 region is copied into the 5'-LTR of the proviral DNA as result of reverse transcription and integration. The vector is thus self-inactivating in that it prevents its further propagation by preventing the transcription of further retroviral genomes from the integrated proviral DNA. The consequence of this wished characteristic is that no stable producer cell lines can be generated by

transduction with a SIN vector.
Thus, starting from the closest prior art **D1** providing already the feature of "high-titre" production of retroviruses (see D1, Table 2) defined by the Applicant to be at least 10^6 retroviral particles/ml, the skilled person faced with the objective problem of the invention (see point 2.3) needed only to introduce into the master producer cell that feature conferring the wished SIN- or REG-characteristic to the retrovirus to be produced in the derived producer cell, said feature being known to the skilled person to be a SIN- or REG-modified U3 region that will be copied into the 5'-LTR of the DNA provirus in a next round of reverse transcription in the target cell.

The preferred embodiments of the dependent claims are different combinations of features present singularly or in combination in the prior art (**D2-D4**), such combinations being also obvious for the person skilled in the field.

3. Industrial applicability (Article 33(4) PCT)

3.1 For the assessment of the present claims 40 and 41 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4. The following remarks are made under Article 6 PCT:

4.1 Claim 10 defines particular features of an "internal 5'-LTR" and refers back to any preceding claim, however the technical feature "internal 5'-LTR" is mentioned for the first time in claim 8.

4.2 Independent claim 18 and its dependent claim 19 both mention a "second NOI". However, a "first NOI" is mentioned for the first time in dependent claim 20 as a feature which is finally not present in the cell. Similarly, claim 24 defines features of a "first NOI" and refers to claim 22, which itself refers back to claims 18 and 19, where a "first NOI" is not mentioned.

In general, the fact that the claimed derived producer cell is characterized in part by concrete final technical features and in part as product by process (by mentioning transient technical features of the vectors used in the 2-step process of its production) is very confusing. The Applicant is reminded that claims have to be clear and concise, meaning also that the reader should be able to understand the invention from the wording of the claim. If a product by process definition is not avoidable, the claimed subject matter should at least be clearly defined in its entirety in an independent claim.

4.3 Although produced by the process of claim 30, the regulated retroviral vectors of claims 35-38 have as technical features only a 5'-LTR, a NOI and a regulatable 3'-LTR, which do not render them novel over the regulated vectors of the prior art (see point 1.1 under novelty). Indeed, the actual broad formulation of the method of claim 30 which does not specify the location of the recombinase sites, includes the production of regulatable retroviruses from derived producer cells having no recombinase site within the provirus, e.g. by introducing a complete floxed proviral unit into a preexisting cellular recombinase site during step ii) of the method.
For the same reason, claim 39 claims virtually any cell of the prior art transduced with any regulated retroviral vector.

4.4 Claim 50 contains unallowable references to the description (R 6.2(a) PCT; PCT Guidelines III 4.10) which engender a problem of novelty (see 1.2). Furthermore, the word "substantially" is vague and unclear in the sense of Article 6 PCT (PCT Guidelines CIII 4.5a).

CLAIMS

1. A method of modifying a producer cell which producer cell comprises integrated into its genome a provirus which provirus comprises one or more recombinase recognition sequences within or upstream of its 3' LTR, the method comprising:
introducing into the cell a construct comprising a 5' recombinase recognition sequence, an LTR (construct LTR) and a 3' recombinase recognition sequence in that order, in the presence of a recombinase which is capable of acting on the recombinase recognition site(s) such that the nucleotide sequence between the 5' and 3' recombinase recognition sequences in the construct is introduced into the provirus, wherein on introduction of said nucleotide sequence into the provirus, said provirus comprises a packaging signal, and the construct LTR is downstream of a 5' LTR within the provirus, said construct LTR being different to said 5' LTR.

2. A method according to claim 1 wherein the construct further comprises at least one nucleotide sequence of interest (NOI) between the 5' recombinase recognition sequence and the construct LTR, which NOI is operably linked to a transcriptional regulatory sequence.

3. A method according to claim 1 or claim 2 wherein the construct further comprises the 5'LTR and/or the packaging signal.

4. A method according to any one of claims 1 to 3 wherein the construct LTR is a heterologous regulatable LTR.

5. A method according to claim 4 wherein the regulatable LTR comprises an ischaemic like response element (ILRE).

6. A method according to any one of claims 1 to 3 wherein the construct LTR is inactive.

7. A method according to any one of the preceding claims wherein the provirus comprises an NOI encoding a selectable marker, which NOI is flanked by recombinase recognition sites

8. A method according to any one of the precedings claims wherein the provirus comprises an internal 5' LTR upstream of the recombinase site or the 5' recombinase site where there is more than one site.

9. A method according to claim 8 wherein the U3 region of the 5' LTR is inactive.

10. A method according to any one of the preceding claims wherein the U3 region of the 5' LTR and/or the U3 region of the second internal 5'LTR comprises a heterologous promoter.

11. A method according to any one of the preceding claims wherein the provirus comprises two recombinase recognition sites and as a preliminary step, the recombinase is expressed in a host cell such that the nucleotide sequence present between the two sites is excised.

12. A method according to any one of the preceding claims wherein the producer cell is a high titre producer cell, capable of producing at least 10^6 retrovirus particles per ml.

13. A method according to any one of the preceding claims wherein the provirus is a lentivirus.

14. A method according to claim 13 wherein the lentivirus is HIV or EIAV.

15. A method according to any one of claims 2-14 wherein the provirus further comprises a second NOI.

16. A producer cell obtainable by the method of any one of claims 1 to 15.

17. An infectious retroviral particle obtainable from the producer cell of claim 16.

18. A derived producer cell comprising integrated into its genome a retroviral vector comprising in the 5' to 3' direction a first LTR (5' LTR); a second NOI operably linked to a second LTR (regulatable 3' LTR) and a third LTR (3'LTR); wherein the third LTR is positioned downstream of the second LTR in the producer cell.

AMENDED SHEET

19. A producer cell according to claim 18 wherein the first LTR comprising 5'R and 5' U5 sequences is derived from a first vector; the second NOI operably linked to a second LTR is derived from a second vector; and the third LTR is derived from the first vector.

20. A producer cell according to claim 19 wherein the first vector comprises a retroviral vector wherein the retroviral vector comprises a first NOI flanked by recombinase recognition sequences.

21. A producer cell according to claim 20 wherein the retroviral vector further comprises an internal LTR located upstream of the first NOI and downstream of a packaging signal wherein the internal LTR comprises a heterologous U3 sequence linked to heterologous R and U5 sequences.

22. A producer cell according to any one of claims 18 to 21 wherein the third LTR is transcriptionally quiescent.

23. A producer cell according to claim 22 wherein the third LTR comprises a deletion in the U3 sequence.

24. A producer cell according to any one of claims 20 to 23 wherein the first NOI is a selectable marker.

25. A producer cell according to claim 19 wherein the second vector comprises a second NOI operably linked to a second LTR (regulatable 3' LTR) comprising at least one recombinase recognition sequence.

26. A producer cell according to 25 wherein the second LTR comprises a deletion in the U3 sequences in the 3'LTR.

27. A producer cell according to claim 25 or claim 26 wherein the second NOI comprises a coding sequence operably linked to a promoter.

28. A producer cell according to claim 27 wherein the second NOI comprises a discistronic construct.

29. A producer cell according to claim 28 wherein the discistronic construct comprises a therapeutic gene, an internal ribosomal entry site (IRES) and a reporter gene.

30. A method for producing a high titre regulatable retroviral vector, the method comprising the steps of:

(i) providing a derived producer cell comprising integrated into its genome a first vector;

(ii) introducing a second vector into the derived producer cell using a recombinase assisted method;

wherein the derived producer cell comprises a retroviral vector comprising in the 5' to 3' direction a first LTR (5' LTR); a second NOI operably linked to a second LTR (regulatable 3' LTR); and a third LTR (3'LTR); wherein the third LTR is positioned downstream of the second LTR in the derived producer cell.

31. A method according to claim 30 wherein the third LTR is transcriptionally active but expression is directed away from the second LTR.

32. A method for introducing a second regulatable 3'LTR into a derived producer cell wherein the method comprises a recombinase assisted method.

33. A method according to claim 32 wherein the recombinase assisted method is a Cre/lox recombinase method.

34. A process for preparing a regulated retroviral vector as defined in claim 17 comprising performing the method according to any one of claims 30 to 33 and preparing a quantity of the regulated retroviral vector.

35. A regulated retroviral vector produced by the process according to claim 34.

AMENDED SHEET

36. A regulated retroviral vector according to claim 35 wherein the retroviral vector is capable of transducing a target site.

37. A regulated retroviral vector according to claim 36 wherein the retroviral vector is produced in sufficient amounts to effectively transduce a target site.

38. A regulated retroviral vector according to claim 36 or claim 37 wherein the target site is a cell.

39. A cell transduced with a regulated retroviral vector according to claim 38.

40. Use of a regulated retroviral vector according to any one of claims 35 to 38 in the manufacture of a pharmaceutical composition to deliver an NOI to a target site.

41. Use of a regulated retroviral vector according to any one of claims 35 to 38 in the manufacture of a medicament for diagnostic and/or therapeutic and/or medical applications.

42. Use of a recombinase assisted mechanism to introduce a regulated 3'LTR into a derived producer cell line to produce a high titre regulated retroviral vector.

43. A derived stable producer cell capable of expressing regulated retroviral vectors according to claims 35 to 38.

44 A derived stable producer cell according to claim 43 wherein the regulated retroviral vector is a high titre regulated retroviral vector.

45. A nucleic acid vector comprising a 5' recombinase recognition sequence, a regulatable LTR and a 3' recombinase recognition sequence in that order.

46. A nucleic acid vector according to claim 45 further comprising at least one NOI between the 5' recombinase recognition sequence and the regulatable LTR.

47. A nucleic acid vector according to claim 45 or claim 46 further comprising a 5' LTR and/or a packaging signal.

48. A nucleic acid vector according to any one of claims 45 to 47 wherein the LTR is a heterologous regulatable LTR.

49. A nucleic acid vector according to any one of claims 45 to 47 wherein the LTR is transcriptionally quiescent.

50. A method and/or a producer cell substantially as described herein and with reference to Figures 2 to 6 and 8 to 17.

AMENDED SHEET